

Whitman (C. O.)

al

THE

Kinetic Phenomena of the Egg during
Maturation and Fecundation.
(Oökinesis.)

BY

C. O. WHITMAN, ✓

DIRECTOR OF THE LAKE LABORATORY, MILWAUKEE, WIS.



presented by the author —

REPRINTED FROM THE JOURNAL OF MORPHOLOGY,

VOL. I, NO. 2, DECEMBER, 1887.

BOSTON:
GINN AND COMPANY.



THE

Kinetic Phenomena of the Egg during Maturation and Fecundation. (Oökinesis.)

BY

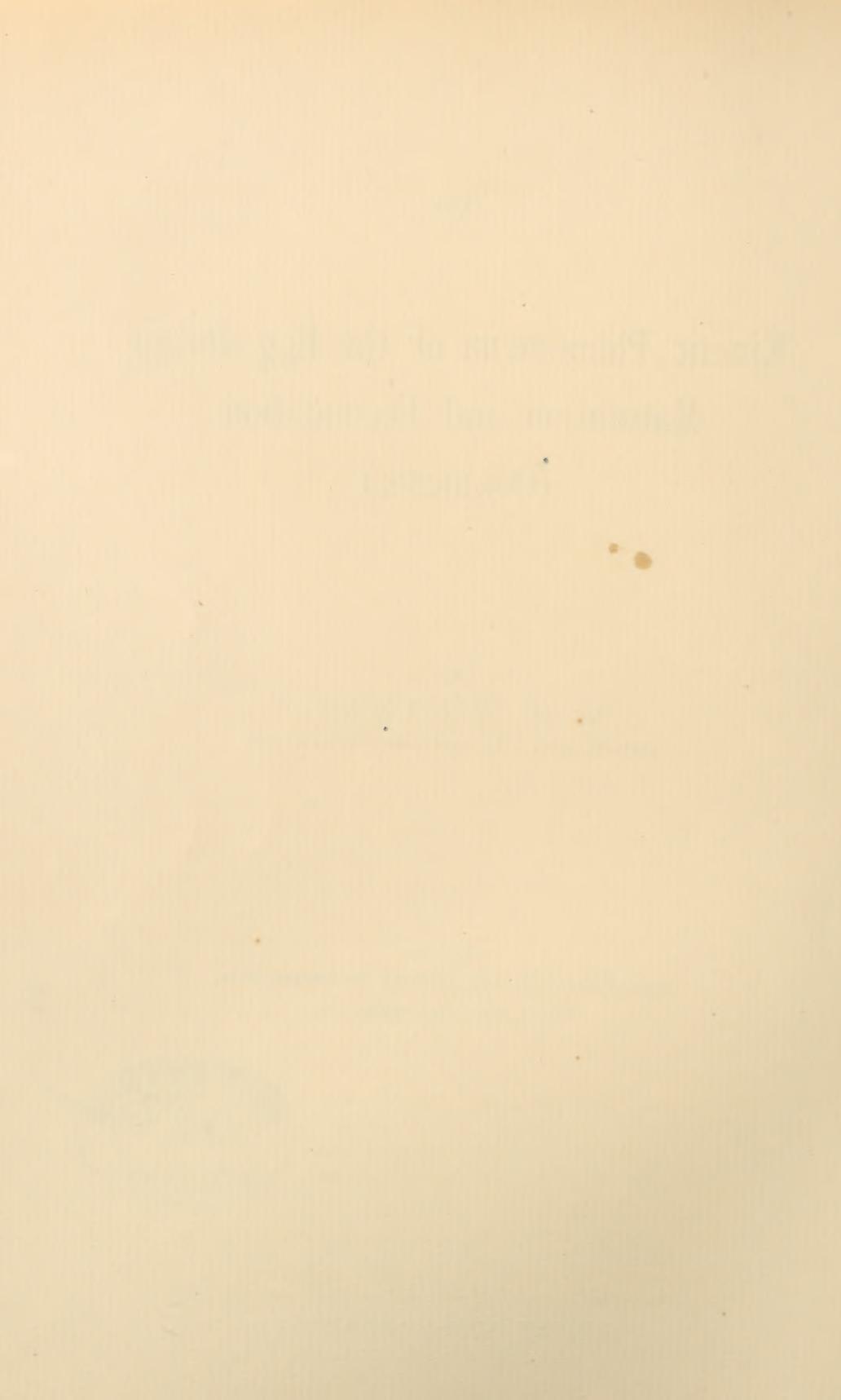
C. O. WHITMAN,

DIRECTOR OF THE LAKE LABORATORY, MILWAUKEE, WIS.

REPRINTED FROM THE JOURNAL OF MORPHOLOGY,
VOL. I., NO. 2, DECEMBER, 1887.



BOSTON:
GINN AND COMPANY.



JOURNAL
OF
MORPHOLOGY.

OÖKINESIS.

C. O. WHITMAN.

THE period of maturation and fecundation of the egg is pre-eminently one of kinetic phenomena. We have here two complex series of events, which together form the prelude to development. Though overlapping, and blending at the point where development begins, they must, nevertheless, be regarded as distinct, inasmuch as maturation, at least in cases of parthenogenesis, may be completed quite independently of fecundation.

The phenomena of maturation embrace the closing chapter in the history of the germinal vesicle, and such concomitant changes in the vitelline protoplasm as prepare the egg for the reception of the spermatic element. The phenomena of fecundation¹ embrace the history of the pronuclei, and those attendant changes in the protoplasm which form the concluding steps in the premorphological organization of the egg. The first series culminates in the production of polar globules; the second, in the formation of the cleavage-nucleus by the union of the pronuclei. In each series we recognize two factors; namely, *nuclei* and *cell-protoplasm*.

To what extent these factors act independently, and how far

¹ The distinction proposed by E. Van Beneden (*Arch. de Biol.*, IV., p. 283) between the *copulation* of the sexual cells and *fecundation* is here adopted.

they influence or react upon each other, are questions still awaiting decisive answers. The natural boundary line between the two, at first clearly defined, begins to fade almost simultaneously with the earliest appearance of kinetic changes, becomes rapidly effaced as the energy of display increases, and is resumed only at regularly recurring epochs, when outward manifestations of activity cease. Under such conditions, with karyoplasm passing uninterruptedly into cytoplasm, it is certainly very difficult, and perhaps quite impossible with the micrographical means at our command, to determine precisely the part played by each.

The majority of writers are inclined to seek the *primum mobile* in the nucleus, and to make the nucleus responsible for the kinetic phenomena displayed in the cytoplasm. Attempts have been made to settle this question experimentally, through the artificial division of infusoria; but thus far no one has undertaken a critical analysis of the phenomena of maturation and impregnation, with a view to finding test cases. It is this side of the subject which I propose to consider in the present paper.

It may be noticed, first of all, that the phenomena in question are not all of an active nature. Some are plainly induced by outside influences, or are simply secondary effects resulting from altered internal conditions. The rapid clearing up of a pelagic fish egg the moment it comes in contact with water, owing to the dissolving of its opaque granules, is an example of this kind. All changes in the relative position of the constituent elements of the egg that result from differences of specific gravity may be referred to the same category. We have, then, to distinguish between active and passive changes, and the latter can be set aside as unimportant to the inquiry. The former may be conveniently divided into two classes, one of which I shall designate *cytokinetic*, in distinction from the other, which is now generally called, after Schleicher's example, *karyokinetic*.¹

¹ The term *karyokinesis* has been objected to by Flemming (Zellsubstanze, Kern und Zelltheilung, p. 376) as neither describing the form nor indicating the nature of nuclear metamorphoses. *Karyomitosis*, or simply *mitosis*, is the substitute proposed by Flemming. Priority and general usage are in favor of *karyokinesis*; besides, this term commends itself, in my opinion, as the simpler and more comprehensive, and as expressing better the essence of the phenomena. Its leading idea is *motion*, but motion viewed as an exponent of forces residing in, or acting upon, the nucleus. It

The whole series of movements and form-changes, progressive and regressive, through which the nucleus passes in the process of division, together with all the kinetic changes displayed in the germinal vesicle and pronuclei, are karyokinetic phenomena.

The phenomena which may be regarded as oökinetic, or cytokinetic, display themselves in the vitelline protoplasm and in the cytoplasm of cells in general. *They are diversiform in the extreme, rarely presenting regular form-series, and thus stand in marked contrast with nuclear metamorphoses, which everywhere, both in plant and animal cells, exhibit a most remarkable uniformity.* This irregularity makes it quite impossible, in the present state of our knowledge, to formulate, or express in general terms, the phenomena embraced under this head.

I. MOVEMENTS OF THE GERMINAL VESICLE AND PRONUCLEI.

The unique character of many of these cytokinetic displays appears to me incompatible with the idea that they are the direct effect of nuclear influence. Any changes in the protoplasm, induced and sustained at the expense of changes taking place in the nucleus, should be as regular and uniform as the karyokinetic processes themselves. On any hypothesis that refuses to admit that the cytoplasm is endowed with subtle powers of its own, and capable of automatic as well as responsive action, how can we account for the characteristic difference between telolecithal and centrolecithal eggs? By what power are the passive yolk-elements restrained from taking the position which they would assume under the influence of gravitation alone? What force drives the germinal vesicle from the centre

regards the nucleus as a seat of energy, which displays itself in phenomena of motion.

Mitosis is at best only a synecdochial expression, in which a part is put for the whole. Allowing that the form-changes of the chromatic loops can be thus characterized, it is evident that the movements of the achromatic elements are entirely ignored. But, even in this limited sense, the word is not free from objection. Flemming defines it as "thread-metamorphoses;" but Carnoy (*La Cellule*, III., p. 319) points out that, etymologically interpreted, it signifies "reduction to thread." Now, it is during kinesis, or mitosis, as Carnoy justly remarks, that the chromatin of the nucleus loses its thread-like form, breaking up into loops or rods, and resuming its filoid aspect only after the division is completed.

to the periphery of the egg during the period of maturation? As this centrifugal movement may be upward, downward, or to one side, even in the same class of eggs (*e.g.*, Teleostei), it cannot be said to be controlled by gravitation, nor can it be purely automatic.

How are we to explain that remarkable centripetal movement of the pronuclei which always forms the concluding step in the arrangements preparatory to development? As is well known, these bodies are invariably formed at or near the surface of the egg, sometimes near the same pole, and at other times at opposite poles. Any attraction assumed to exist between them would only be competent to account for their coming together, but would afford no explanation of their centripetal movement. The direct influence of gravitation can no more account for this than for the centrifugal movement of the germinal vesicle. We are driven to the conclusion that the phenomenon is due to the *interaction of nuclear and cytoplasmic forces*. It is not admissible to assume that either factor is passive, but rather that each acts and reacts upon the other. It is by virtue of this subtle interaction (Wechselwirkung) that the pronuclei ultimately assume a position of equilibrium with respect to the active constituents of the oöplasm.

But will the attraction which we must assume to exist between the oöplasm and the pronuclei account for all that we know about the behavior of the latter? Or are we under the necessity of assuming still another attraction acting between the pronuclei themselves? The majority of writers would answer unhesitatingly the first question in the negative, and the second in the affirmative. While I fully concur in this opinion, it seems to me that the reasons generally assigned for it require examination.

The formation of asters in connection with the pronuclei, and the fact that these bodies approach each other and eventually unite at the centre of the egg, or at some point which, though not the geometric centre, yet represents the virtual centre of their sphere of action, are commonly regarded as proof that they attract each other. If we analyze these facts, we shall find that they afford very little evidence in support of this view.

The astral displays demonstrate action of some unknown kind between the oöplasm and the pronuclei, but they give no posi-

tive indication of any action whatever between the two pronuclei. Now this action, which we may call *centripetal attraction* for want of a more definite term, is, as before stated, all that is required to account for the centripetal movement of the pronuclei. But would this movement result in bringing the pronuclei together? Evidently it would, if the attraction was the same for each pronucleus.

Wherein, then, lies the evidence of attraction between the pronuclei? In order to elucidate this question let us consider how such a force would manifest itself in the movements of these bodies. We should have two forces, one tending to bring the pronuclei together in a direction marked by the chord joining them, the other tending to draw them along their respective radii to the centre of the egg. The place of meeting and the nature of the lines described by the pronuclei will depend on a variety of circumstances. Prominent among these are the *place* and *time* of origin. The probability that neither of these forces acts uniformly throughout, and the possibility that the attraction between the pronuclei may act only within shorter distances than those by which these bodies are often separated, will have to be taken into account.

To begin with, let us suppose that the pronuclei are contemporaneous in origin and located at opposite poles. A typical illustration of such conditions is furnished in the nematode egg. The component forces would here act in the same direction and drive the pronuclei straight to the point of meeting (*c*). The possibility of meeting at the centre would depend upon the obvious conditions,—(1) that they start at the same moment and move with equal velocity, or (2) that any difference in one of these respects is neutralized by a counter difference in the other.

If the starting-points (*m* and *f*, Fig. 2) were near the same pole, the other conditions remaining the same, the place of meeting (*x*) would be eccentric, and the nature of the paths described would, of course, depend on the relation sustained between the component forces. If this relation is uniform, the pronuclei will move in *straight* lines to the

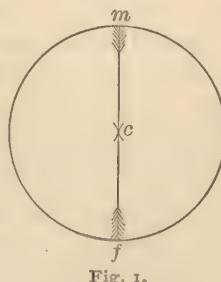


Fig. 1.

point of meeting (x'), and then along the radius in which this point lies to the centre (c). If, on the other hand, it varies, it may do so in many ways, three of which may be noticed here.

Allowing, what seems most probable, that the centripetal attraction steadily diminishes, the zero-point being reached at the centre, we may assume (1) that the nuclear attraction remains constant; or (2) that it increases as the distance between the pronuclei diminishes; or (3) that, acting only at relatively short distances, it does not come into play until the centripetal migration is partially completed. In the first

two cases the paths of the pronuclei would be represented by curves, with the concave sides towards the pole, with the difference only that the curves would be stronger in the second case than in the first. In the third case the paths would continue straight until nuclear attraction began to act, and then curve towards the pole until the point of junction was reached. In all three cases the course after meeting would be centripetal along the radius in which the point x (Fig. 2) lies. Essentially the same thing would happen in a telolecithal egg, except that the point c , representing the centre of equilibrium rather than the geometric centre, would be nearer the active pole.

In the movements thus far considered there are only two peculiarities, the occurrence of which could be regarded as conclusive evidence of nuclear attraction; namely, the *curved* paths of the pronuclei (Fig. 2), and their meeting *before* reaching the centre. The first of these peculiarities is remarkably well shown in the amphibian egg (1, 2, 3, 4), where the path of the male pronucleus is plainly marked by a streak of pigment concave towards the dark pole; and the second has been repeatedly

1. OSCAR HERTWIG. Beiträge zur Kenntniss der Bildung, Befruchtung und Theilung des thierischen Eies. *Morph. Jahrb.*, III., 1877.

2. CHARLES VAN BAMBEKE. Recherches sur l'Embryologie des Batraciens. *Bull. de l'Acad. roy. de Belgique*, LXI., 1876.

3. Id.—Sur les trous vitellins que présentent les œufs fécondés des Amphibiens. *Bull. de l'Acad.*, etc., 2e sér., t. XXX., 1870.

4. WILHELM ROUX. Beiträge zur Entwicklungsmechanik des Embryo. *Arch. f. mik. Anat.*, XIX., H. 2, Pl. x., 1887.

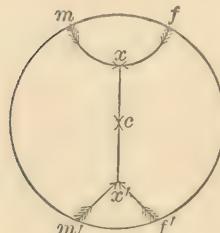


Fig. 2.

observed by Hertwig (5), Fol (6), Selenka (p. 87), Mark (8), and others.

For the sake of simplicity, we have proceeded thus far on the assumption that the pronuclei are contemporaneous in origin, and that they begin to migrate simultaneously. But the conditions will agree more nearly with those generally occurring in nature, if we represent them as varying considerably both in the time of origin and the time of starting. The slight advantage in respect to starting-point, which the female pronucleus has over the male pronucleus, and the velocity of movement, can be left out of account in considering the influences that affect the course and direction of migration.

The time of starting is the chief source of the variations which we have now to consider. The difference in this respect is carried to the extreme in those cases where one of the pronuclei reaches its destination before the other is ready to begin its march. In such cases the earlier pronucleus, starting from any point (as f or f') in the periphery, would be carried, by centripetal attraction alone, in a straight line to the centre (c). From this point it would then advance along another radius to meet the later pronucleus at some point (as x); and, after meeting, the united pronuclei would move back to c . The centrifugal movement of the early pronucleus from c to x would be slower than the centripetal movement of the later pronucleus from m to x , since the former movement would represent the *difference*, and the latter the *sum* of the same two forces. Precisely similar cases of centrifugal movement have been observed, and they furnish another decisive proof of nuclear attraction.

A capital illustration is furnished in the egg of *Toxopneustes lividus*. After saying that the male pronucleus leaves the

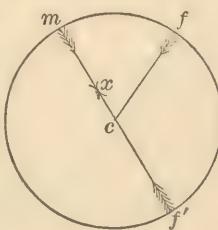


Fig. 3.

5. OSCAR HERTWIG. Beiträge z. Kenntniss d. Bildung, Befruchtung u. Theilung d. thier. Eies. *Morphol. Jahrb.*, I., 1876, p. 381.

6. HERMANN FOL. Recherches sur la Fécondation et le Commencement de l'Hénogénie, 1879, p. 259.

7. EMIL SELENKA. Befruchtung des Eies von *Toxopneustes variegatus*. Leipzig, 1878.

8. EDWARD L. MARK. Maturation, Fecundation, and Segmentation of *Limax campestris*. *Bull. Mus. Comp. Zool.*, VI., 1881, p. 222.

periphery of the egg with "wahrnehmbarer Geschwindigkeit," travelling in the direction of the female pronucleus ("Eikern"), Hertwig (5, p. 380) remarks as follows: "Während dieser so beachtenswerthe Vorgang sich abspielt, verharrt der Eikern nicht in Unthätigkeit; vielmehr setzt sich derselbe gleichfalls in Bewegung, sobald als die Radienfigure [male pronucleus] von der Oberfläche sich entfernt, und rückt näher nach der Eimitte zu. Doch ist seine Bewegung *langsam* und kann leicht übersehen werden, wenn man nicht ein Object gewählt hat, in welchem der Eikern recht peripher gelagert ist. Dass aber eine Bewegung stattfindet, davon habe ich mich ganz sicher überzeugt, indem ich die Lageveränderung des Kerns mit dem Mikrometer controlirte.

"Das resultat dieser Vorgänge ist, dass beide Körper endlich sich treffen entweder in der Eimitte oder wenigstens in der Nähe derselben. *In letzterem Falle verändern dann dieselben noch nachträglich zusammen allmälig ihre Lage, bis sie das Eicentrum einnehmen.*"

With reference to the same point, Fol (6, p. 259) says: "L'attraction se manifeste bien plus vivement sur le pronucléus mâle que sur l'autre noyau, puisque ce dernier ne commence à se mouvoir et à se déformer que lorsque le noyau mâle arrive presque à le toucher."

According to both Hertwig and Fol it is the *male* pronucleus that moves with the greater velocity. But if the explanation I have offered is correct, it depends upon the *time of formation*, which pronucleus will make the longer journey, and which will move the more rapidly. If the male pronucleus reaches the centre of the egg before the female pronucleus is formed, the former will travel the longer distance, but the latter, when it begins to move, will advance with the greater velocity.

A full confirmation of this view is furnished by the observations of Van Beneden (9) on *Ascaris megaloccephala*; and it is all the more satisfactory as this investigator evidently had not directed his attention to the points under discussion. First of all we have the important fact (confirmed by Zacharias, 10) that

9. ED. VAN BENEDEIN. *Recherches sur la maturation de l'œuf et la fécondation.* *Arch. de Biol.*, IV., 1883.

10. OTTO ZACHARIAS. *Neue Untersuchungen, etc.* *Arch. f. mik. Anat.*, XXX., H. I., p. 149, 1887.

the male pronucleus reaches the centre of the egg before the female pronucleus is formed, as shown in the following statement: "Le spermatozöide, modifié comme je l'ai exposé plus haut, occupe pendant toute la période qui se termine par l'expulsion du second globule polaire et la libération de la seconde couche périvitelline *le centre géométrique de l'œuf*" (p. 489). Moreover, Van Beneden states that, in order to meet the male pronucleus, the female pronucleus traverses the greater distance, and hence, by inference, moves the more rapidly: "Pour se rejoindre à son congénère le pronucléus femelle, qui prend toujours naissance au voisinage du pôle supérieur de l'œuf, parcourt un chemin beaucoup plus long que le pronucléus mâle; celui-ci se déplace relativement peu" (p. 524).

In saying that the female pronucleus makes a longer journey than the male pronucleus, the author evidently wholly ignores the distance previously traversed by the latter in reaching its central position.¹

We find, then, three facts, which can be said to furnish indisputable evidence of attraction between the pronuclei. These are: —

1. The *curved* path of the male pronucleus in the amphibian egg.
2. The *meeting* of the pronuclei *before* reaching the centre of equilibrium.
3. The *centrifugal* movement of the *earlier* pronucleus to meet the *later* formed pronucleus.

That this attraction acts only at comparatively short distances, and not at all distances which may separate the places of origin of the pronuclei, is demonstrated by the following very interesting observation of Fol's (6, p. 106): "A mesure que l'aster mâle s'avance dans le vitellus, ses rayons deviennent toujours plus longs et plus accentués; sa liaison avec son point d'origine à la surface du vitellus se perd. *Sa direction, d'abord*

¹ It will probably occur to any one tolerably familiar with Van Beneden's paper, that he holds that the pronuclei are contemporaneous in origin, while the validity of my explanation rests upon a contrary assumption. The contradiction is, however, only apparent, not real; for it is one of words rather than facts. The difference is accounted for by the fact that Van Beneden maintains that the spermatozoon undergoes a sort of maturation after penetrating the ovum, and that the changes through which it becomes a proper pronucleus take place only *after* it has reached the centre, simultaneously with the formation of the female pronucleus.

centripète, change, lorsque le pronucléus femelle n'occupe pas le centre de l'œuf, pour se rapprocher de ce dernier noyau. Enfin le pronucléus femelle, jusqu'alors immobile se met en mouvement au moment où il est atteint par les rayons de l'aster mâle, et la réunion des deux noyaux s'opère promptement.

The same fact is brought out most clearly by the researches of Wilhelm Roux (4) on the frog's ovum. Roux's observations are all the more conclusive, as he has made a special study of the movements of the pronuclei, with a view to determining the precise axial relations of the paths described. The spermatic body takes at first a centripetal direction, penetrating to a depth of .29-.35 mm., and thus describing the "*penetration-path*." By a more or less abrupt curve the path then becomes directed towards the female pronucleus, — becomes nucleopetal, — and is thenceforth called the "*copulation-path*." The angle formed by the first and second parts of the course varies from 90° to 180° , according to the distance of the point of penetration from the pole. The first part of the course is accomplished through a simple "*movement of penetration*;" the second, under the influence of nuclear attraction. Roux does not undertake to assign any reason for the change from a centripetal to a nucleopetal direction, but alludes to the possibility that the nucleopetal course sets in when the transformation of the spermatozoon into the male pronucleus becomes complete.

Centripetal attraction, as we have seen, may, under certain conditions, act entirely alone upon one or both pronuclei; but the attraction between the pronuclei must act, under all normal conditions at least, concomitantly with centripetal attraction. I am aware that the language often used in describing the movements of the pronuclei is not in full accord with the last statement. Both Hertwig and Fol seem to be very strongly impressed with the idea that the male pronucleus must, under all circumstances, move more rapidly than the female pronucleus; and both declare that the path taken by the pronuclei, as they advance to meet each other, is represented by a single straight line, instead of two straight or two curved lines meeting at an angle, as here maintained. In one of his latest papers Hertwig (11, p. 281) expresses himself as follows: "Wic nun

11. OSCAR HERTWIG. Das Problem der Befruchtung und der Isotropie des Eies. *Jenaische Zeitschrift*, XVIII., 1885.

früher der Samenfaden das Ei aufgesucht hat, so wandert jetzt der Spermakern *in gerader Richtung dem Eikern entgegen*, welcher sich gleichfalls, wenn auch *viel langsamer*, in Bewegung setzt." Fol (6, p. 259) puts it thus: "Ainsi lorsque le noyau femelle se trouve dans une position excentrique et que le noyau mâle prend naissance près de ce dernier, il marche directement à sa rencontre *suivant une corde de cercle* au lieu de se rendre d'abord au centre du vitellus."

These descriptions would be strictly accurate if our two forces acted consecutively and not concomitantly; *i.e.*, if nuclear attraction acted alone before the meeting of the pronuclei, and centripetal attraction alone after this event. It is possible — and this observation of Fol makes it highly probable — that the attraction between the pronuclei is much stronger than the centripetal attraction. We know of no fact that forbids this supposition, and, if this point be conceded, the difficulty of reconciling the observation with our views practically disappears.

That what we have called centripetal attraction is a reciprocal action between oöplasm and pronuclei is a conclusion supported by still another interesting fact. In the centrifugal march of the germinal vesicle and in the maintenance of a peripheral position by the archiamphiaster during the production of the polar globules, there is satisfactory evidence, as Fol has already pointed out, of a *repellent action*. In those cases where the spermatozoon penetrates the ovum before the elimination of polar globules (Teleostei, Nematoidea, Hirudinea, etc.), we have attraction and repulsion exhibited at one and the same time, and the oöplasm is the *common factor* in both actions. It is thus made evident, first, that the body attracted and the body repelled cannot be identical in molecular constitution; and second, that the two modes of action are due to the unlike physico-chemical relations which these bodies respectively sustain towards the oöplasm.

Allowing that both the attraction and the repulsion represent reciprocal action, we are brought face to face with the question of the relative importance of the two factors engaged in each case. Do the points just noticed throw any light on this question? I believe they furnish at least one very important evidence in support of the opinion that the nucleus takes the initiative in action.

Up to a certain time we see the germinal vesicle held in place by centripetal attraction; then, owing to unknown changes either in itself or in the oöplasm, or in both, repellent action sets in, and it begins to move centrifugally. Now, if it can be shown that the conditions of centripetal attraction remain unchanged, so far as the oöplasm is concerned, the changes which induce repellent action must evidently be located in the germinal vesicle. That no changes take place in the oöplasm which can interrupt the action of centripetal attraction is shown by the deportment of the male pronucleus at the very time when the expulsion of polar globules is in progress, and by the fact that this attraction acts alike¹ on both pronuclei, irrespective of the time, place, or order of their origin. All the essential conditions of centripetal attraction in the oöplasm may then be said to be unaffected by the processes of maturation and fecundation. The primary cause of centrifugal movement must, therefore, lie in the germinal vesicle itself. To ascertain the nature of the causal changes is a task of the future.

Various causes have been assigned for the centrifugal movement of the germinal vesicle. In the meroblastic eggs of vertebrates this movement is connected with the formation of the *latebra* (Purkinje); and in the holoblastic eggs of the Amphiibia, with the origin of the "*figure claviforme*" (Bambeke). The fact that this movement in the hen's egg follows so closely upon the appearance of the white yolk spherules, might raise suspicion of a passive displacement, the germinal vesicle being pushed upward by the formation of the yolk elements beneath it. This view, advanced by Van Beneden (12, p. 206), is com-

¹ It cannot perhaps be said that in all cases this force acts with *equal intensity* on both pronuclei. In *Toxopneustes variegatus*, for instance, Selenka ("Befruchtung des Eies, etc.," Leipzig, 1878) states that the "Eikern" (female pronucleus) always takes an eccentric position (p. 4), and there remains until after the formation of the male pronucleus. The male pronucleus, on the contrary, makes no delay in its centripetal movement, and, having gained the centre, awaits there the approach of the female pronucleus, which does not appear to move until reached by the astral rays of the former (pp. 7-8). It would appear from Selenka's description that the Eikern is drawn from its eccentric position by nuclear attraction alone. In exceptional cases the spermatozoon penetrates the egg in the immediate vicinity of the Eikern, and then the two pronuclei unite and move slowly towards the centre (p. 8). Selenka makes no mention of a centrifugal movement on the part of the male pronucleus.

12. ED. VAN BENEDEEN. Recherches sur la composition et la signification de l'œuf. *Mem. cour. d. l'Acad. roy. des Sciences de Belg.*, XXXIV., 1870.

pletely disproved by the mode of origin of the latebra (13, p. 67). Oellacher's (14, pp. 18, 24) theory of the expulsion of the germinal vesicle by contractions of the vitellus has been disposed of by more recent observations.

The idea of passive displacement has recently been amplified to a general theory by Ryder (15, pp. 95-101). When we reflect that this movement is common to all types of eggs, that in the majority of cases it takes place after the egg has attained its full size, and after the formation of the food-yolk is completed, we find it difficult to accept this mode of explanation, and quite impossible to concede that the "*law of displacement*" is to be found in this direction. The holoblastic eggs mentioned by Ryder (p. 101), not to mention numerous others, furnish evidence quite fatal to his theory. Take the single example of the frog's egg, where the germinal vesicle maintains a nearly central position until a short time before deposit, taking up its centrifugal march after the food-yolk is all present. If the germinal vesicle is simply crowded to the surface by food-yolk, how can the penetration of the yolk by the pronuclei be accounted for? Equally fatal to the theory is the persistence of the latebra and the cord of protoplasm connecting it with the cicatricula.

II. RECEPTIVITY OF THE OVUM FOR SPERMATOZOA.

The idea seems to have been widely accepted that the accessibility of the ovum to the spermatozoa is regulated by external, mechanical means, rather than by internal, physiological conditions. It is well known that the period of fecundation is, generally speaking, relatively a short one; but the distinction between *receptivity* and *accessibility* is very generally ignored.

If centripetal attraction is persistent, as maintained in the foregoing chapter, why should the ovum enjoy only a transitory receptivity? Can the duration of this period be brought into

13. WILH. WALDEYER. *Eierstock und Ei.* Leipzig, 1870.

14. J. OELLACHER. *Beiträge zur Geschichte des Keimbläschens im Wirbelthiere.* *Arch. f. mik. Anat.*, VIII., 1872.

15. JOHN A. RYDER. "Embryography of Osseous Fishes." *Annual Report of the Commissioner of Fish and Fisheries for 1882.*

definite relation with any of the internal changes before considered? If the view presented in the preceding pages be tenable, the period of receptivity may be said to date, not from the expulsion of the polar globules, but *from the moment the conditions of centripetal attraction are reversed in the germinal vesicle*. A period of non-saturation begins with the centrifugal movement of the germinal vesicle, and terminates with the penetration of the spermatic body. As soon as all the elements of saturation are present, external manifestations of centripetal attraction cease, and there remains only the work of internal equilibration, which ends with the centripetal march of the pronuclei.

From this standpoint, it is idle to talk about mechanical contrivances for preventing the admission of supernumerary spermatozoa, as if the receptivity of the ovum were not self-regulating. The idea that the micropyle may be closed against spermatozoa by a polar globule, as held by Hoffmann (16, p. 68), is inadmissible, as will be shown by observations soon to be published. Allowing Hoffmann's observation to be correct, what grounds have we for supposing that spermatozoa could not pass directly through a polar globule lodged in the micropyle? Has not the penetration of polar globules by spermatozoa been repeatedly observed? And is it at all probable that a polar globule would prove more renitent within the micropyle than elsewhere?

Equally untenable is the suggestion of Calberla (17, p. 458), that the tail of the spermatozoon is left in the micropylar canal for the purpose of blocking the way to other spermatozoa.¹

In the Echinoderm egg, according to Fol (6, p. 94), it is the rapid formation of an impenetrable vitelline membrane, at the moment when the spermatozoon comes in contact with the vitellus, which makes it impossible for other spermatozoa to enter. There is no evidence that superfetation would follow if such a membrane were not formed; hence its formation would not necessarily be connected with any such function as Fol has ascribed to it.

16. C. K. HOFFMANN. *Zur Ontogenie der Knochenfische*. Amsterdam, 1881.

17. E. CALBERLA. *Der Befruchtungsvorgang beim Ei von Petromyzon Planeri*. *Zeitschr. f. wiss. Zool.*, XXX., 1877.

¹ Kupffer and Benecke affirm, positively, that the tail is not left in the micropyle.

Besides, Hertwig (18, p. 173) has shown quite conclusively that this membrane is already present *before* any spermatozoon reaches the vitellus, and concludes that it is not a mechanism for limiting the number of spermatozoa admitted. Hertwig remarks (p. 173), that the existence of membraneless eggs is against the idea advanced by Fol, and adds,—"It seems to me, therefore, that it is the egg-plasma itself which alone, during unimpaired vitality, can prevent the entrance of more than one spermatozoon. At all events, this phenomenon finds its analogue in the copulation of the lowest unicellular plants and animals, where one also sees only two cells uniting in the sexual act."

Selenka (19, p. 4) places himself on the side of Fol.

Kupffer and Benecke (20, p. 21) have advanced the idea that the spermatozoon is drawn into the vitellus by an attractive influence emanating from an egg-nucleus. Mutual attraction between the vitellus and this nucleus carries the latter from its place of origin near the formative pole, towards the centre of the egg; and as the attraction between the nucleus and the spermatozoa diminishes as the distance increases, only the foremost spermatozoon may be supposed to keep within its influence, all the rest being left behind. As this view is plainly incompatible with what is now known about the movements of the pronuclei, it requires no further notice.

We are not infrequently told that, when the spermatozoon enters the egg before maturation is complete, it remains unchanged and inactive in the periphery of the egg until the polar globules are ejected. It is evident, however, that observations on this point have not been sufficiently close and searching in many cases, as often happens when observations are made at random, and in ignorance of their theoretical bearings. The time of appearance of the male aster, in some cases, would raise a suspicion that centripetal attraction manifests itself more strongly after the elimination of the polar globules than before; but I have failed to find a single well-ascertained fact in support

18. O. HERTWIG. Beiträge z. Kenntniss d. Bildung, Befruchtung u. Theilung d. thier. Eies. Dritter Theil. I. Abschnit. *Morph. Jahrb.*, IV., 1878.

19. EMIL SELENKA. "Befruchtung des Eies von *Toxopneustes variegatus*." Leipzig, 1878.

20. KUPFFER and BENECKE. Der Vorgang der Befruchtung am Eie der Neunau- gen. Königsberg, 1878.

of the opinion that, under perfectly normal conditions, the spermatic body remains inactive and stationary at the surface of the egg until the polar globules are excluded.¹

In the case of the teleostean egg, I have positive proof, not only that the spermatozoon enters the egg *before* any polar globule is formed, but that the pronucleus derived from it attains a central position before the female pronucleus begins to move.

In the Hirudinea, Bütschli (21, p. 5) and Hertwig (1, p. 30) failed to find the male aster before the appearance of the first polar globule, and my earlier efforts were equally unsuccessful. I have since succeeded in finding a distinct male aster in the fresh-laid egg of Clepsine, *i.e.*, from thirty to forty minutes before the first polar globule arises. The aster at this time has already penetrated to a depth of one-quarter or even one-half the radius of the egg, and is soon after found at, or near, the centre. I am unable to say positively whether it advances somewhat from this position to meet the female pronucleus, but the appearances indicate a slight movement of this kind. The phenomena of fecundation in the Hirudinea, so far as at present known, accord with the conclusions already set forth.

With reference to *Ascaris megaloccephala*, Oscar Hertwig (11, p. 282) makes the following somewhat surprising remark: "Hier bleiben die grossen Samenkörper, welche die Gestalt einer Spitzkugel haben, längere Zeit nach ihren Eindringen ganz unverändert in ihrer ursprünglichen Gestalt in der Eirinde liegen." The investigations of Van Beneden (9) and Nussbaum (22), cited in support of this statement, unfortunately con-

¹ Fol (No. 24, pp. 106-107) states that in those cases where the egg is fecundated before the formation of the polar globules is completed, the male pronucleus remains at the edge of the vitellus in the condition of a small, immobile, and hardly visible spot until the moment when the elimination of those globules is accomplished. Both pronuclei then arise simultaneously, and each takes its own independent course towards the centre. *The place of meeting, however, is between the centre and the formative pole, because the male pronucleus advances more rapidly.* It is to be remembered that, under normal conditions, the female pronucleus is formed before fecundation; and, further, that the male pronucleus, in the cases referred to, started from, or near, the nutritive pole.

21. O. BÜTSCHLI. *Studien über die ersten Entwicklungsvorgänge der Eizelle, etc.* Frankfurt a. M., 1876.

22. MORITZ NUSSBAUM. "Ueber die Veränderungen der Geschlechtsproducte bis zur Eifurchung." *Arch. f. mik. Anat.*, 1884.

tradict it in both points. On pages 392-394, Van Beneden formally enumerates the changes exhibited in the protoplasmic substance of the spermatozoon the moment it comes in contact with the egg; and on page 395, he distinctly states that the nucleus undergoes, at the same time, "une modification très apparente." Nussbaum's statements are a little less explicit; but, so far as they go, confirm those of Van Beneden. But it is only after the expulsion of the second polar globule, according to Van Beneden (p. 355), that the spermatozoon undergoes "those modifications which announce the imminence of fecundation," and this statement may possibly have misled Hertwig. A citation, previously made (p. 235), from Van Beneden shows that Hertwig was also mistaken in supposing that the spermatozoon remained stationary for a considerable time at the surface of the egg.

Zacharias (10) fully confirms Van Beneden's statements in regard to the centripetal movement of the spermatic body, and gives, besides, a detailed description of the changes which take place in it before the formation of the second polar globule. In unfertilized eggs the first polar globule is formed, but not the second. This fact shows how erroneous is the idea that the spermatozoon remains passive until after the extrusion of the polar globules.

III. THE POLE OF IMPREGNATION.

The copulation of the sexual cells is attended with very interesting oökinetic phenomena. A remarkable example has been described by Fol (6, pp. 91, 249), in the egg of *Asterias glacialis*. The protoplasm rises up at one point in the form of a cone, which continues to elongate until it meets the spermatozoon on its way through the mucous envelope ("oölemma"). The height of the cone depends on the rapidity with which the spermatozoon advances. If it progresses slowly, the cone may attain a height equal to half the thickness of the oölemma. As soon as contact is established, the cone begins to shorten, but rarely disappears entirely. Its summit, terminating in the remnant of the tail of the spermatozoon, usually remains above the surface of the egg, and soon becomes the point of departure for a new cone, — the "cone of exudation," — which is supposed to arise by expulsion of the vitellus.

The point of chief interest here is the fact that attraction between the oöplasm and the spermatozoon can manifest itself *at a distance*. Difficult as it is to explain the mechanism of such action, the fact itself is so conclusively established that we are compelled to accept it. At first sight, the fact appears to stand entirely alone; but there is something very closely analogous in the attraction between the pronuclei. They certainly attract each other at very considerable distances; but it may be a question whether they act directly on each other, or through the medium of the oöplasm which bridges the distance between them. That the action of the pronuclei upon the oöplasm—whether on the hypothesis of currents or that of polar attraction—cannot account for the behavior of these bodies toward each other is conclusively shown by the fact that *supernumerary male pronuclei do not unite, although they do develop astral radiations*. If the influences which manifest themselves in these astral lines are not competent to account for all the movements of the pronuclei, how can we escape the conclusion that the pronuclei act directly on each other?

The necessity of recognizing two distinct kinds of attraction is thus made very clear. On the one hand, we have the direct action of one nuclear body upon another nuclear body, which we have called *nuclear attraction*; and, on the other, the action of nuclear bodies on the oöplasm, which manifests itself in astral lines, and to which we have given the name *centripetal attraction*. The cone of attraction in *Asterias* may be regarded as a manifestation of centripetal attraction under exceptional conditions; for, although the cone moves toward the spermatozoon, this fact does not exclude the idea of reciprocal attraction. Although, from the nature of things, we do not expect to see the egg move, as a whole, towards the spermatozoon, there are, at least, very strong grounds for believing that it attracts at the same time that it is attracted, and that its attractive influence is always felt before actual contact takes place.

I am fully aware that most writers hold that the sexual products are brought together, not by attractive influences, but by the impelling action of the tail of the spermatic body. Fol is so strongly impressed with this belief that he examines all other hypotheses before accepting that of attraction at a dis-

tance. We believe that such attraction is exerted, not only by the spermatozoon, but also by the egg, and that the part it plays in bringing together the sexual cells is no less important than that taken by the tail of the spermatozoon. It is highly probable, also, that *this attraction is polar, and that the place of penetration is a predetermined point or region.*

On this question, however, we have conflicting testimony, which has been considered at some length by Van Beneden (9, pp. 371-376). Fol and Hertwig concur in the opinion that the spermatozoon may penetrate at any point; while Selenka (7, p. 6) holds that, *as a rule, it enters a preformed protuberance of the vitellus* ("Dotterhügel"), but adds that in about a dozen cases out of a hundred, it may effect an entrance at any other point. Van Beneden suggests that this "Dotterhügel" of *Toxopneustes variegatus* (Selenka), and of *T. lividus* (Flemming, 23), corresponds to the "bouchon d'imprégnation" in *Ascaris*; and this view appears to be well taken. It is supported by one very important consideration, not mentioned by Van Beneden. I refer to the evidence of a micropyle, or micropylar region, which may be drawn from the observations of Hertwig, Fol, and Selenka. Hertwig (18, p. 173) has demonstrated clearly that the vitelline membrane is present before the copulation of the sexual products, and Fol (6, p. 94) has obtained equally positive evidence of a small opening ("micropyle d'occasion") located in a crater-like inflection of this membrane. Fol did not, however, recognize any such crater before the spermatozoon entered the vitellus, but he did find the "cone of attraction" before this event, and observed that *the cone remained in continuity with the vitellus "à travers la membrane,"* after the penetration of the spermatozoon. Allowing, then, that both the cone and the membrane exist prior to fecundation, the same must be conceded for the micropyle. Only one way of avoiding this conclusion occurs to me. The cone might be regarded as a portion of the membrane which is thrown into continuity with the vitellus secondarily, as the result of the penetration of the male element. There is an obvious objection, however, to considering the "cone of attraction" as a part of the vitelline membrane. On this very im-

23. W. FLEMMING. Beiträge z. Kenntniss d. Zelle u. ihrer Lebenserscheinungen. *Arch. f. mik. Anat.*, XX., 1882.

portant point Fol's investigations leave us in the lurch; for, while he describes the cone (p. 91) as arising from the cortical layer of hyaline protoplasm (which layer represents the vitelline membrane *ab initio* Hertwig, *in posterum* Fol) he finds himself unable, when he comes to a final discussion of the matter (pp. 250-251), to decide whether the cone forms a part of this layer or a part of the vitellus proper. As there is no doubt expressed about the continuity of the cone with the vitellus *after* the penetration of the spermatozoon, it may be safely inferred that the continuity exists from the outset.

If Fol's theory of the origin of the vitelline membrane were correct,—it is difficult to accept it in the face of Hertwig's observations, which accord so much better with our general knowledge of related phenomena,—the origin of the cone from the cortical layer could easily be reconciled with its continuity with the vitellus, since this entire layer is supposed to be an integral portion of the egg protoplasm up to the moment of impregnation. The delimitation and separation from the vitellus, resulting from impregnation, would take place all around the cone, leaving the cone still continuous with the vitellus. This view finds its strongest support in the fact that when several spermatozoa enter the same egg at different points, as may happen in pathological cases, a cone of attraction is developed at each of these points (p. 119).

Although any point of the egg may give rise to one of these cones, it is still probable that, in all normal cases, the single cone arises from a differentiated place, which corresponds to the Dotterhügel of *Toxopneustes*. When the cone is first seen, it has the form of a low, "nipple-like prominence" (p. 91), like the "Dotterhügel" in the egg of the sea-urchin.

Flemming states that this prominence is quite difficult to find in *T. lividus*, and that it must be carefully searched for by rolling the egg slowly, and examining attentively every point of the surface. It is probable that Fol overlooked this prominence until, at the penetration of the spermatozoon, its exact position was marked and easily brought into the field of vision.

The "Dotterhügel" of Selenka, the "Höckerchen" of Flemming, the "Protoplasmabrücke" of Hertwig (18, p. 173), and the "cône d'attraction" of Fol are, in all probability, identical in origin and function; and Selenka's observations on the

behavior of the spermatozoa towards the "Dotterhügel" go to show that it is really a "bouchon d'imprégnation." Selenka's interpretation of this as a protuberance caused by the extrusion of the polar globules is entirely unsupported by analogy in other eggs; and, besides, it offers no explanation of the decided preference shown by the spermatozoa for entering the egg at this point. This *preference* must mean that the attraction between the egg and the spermatozoon is strongest at the "Dotterhügel." Selenka accounts for it by supposing that the gelatinous envelope of the egg is more easily pierced in the immediate vicinity of the Dotterhügel than elsewhere.

The fact that spermatozoa generally penetrate the envelope *vis-a-vis* the "Dotterhügel" may be quite as readily explained on the hypothesis of attraction; and this view is supported by analogy, as we shall presently see, and by one of Selenka's own observations. In case the spermatozoon passes through the mucous envelope at some point more or less remote from the "Dotterhügel," it does not continue to advance straight through the vitelline membrane ("Rindenschicht"), but "*swims about over the membrane for from one-half to several minutes, until, by its whip-like movement, it accidentally strikes its head against the Dotterhügel*" (p. 6). It then bores its way through the membrane into the vitellus.

The careful investigations of Zacharias have led him to conclude that there is no predetermined point of impregnation in the egg of *Ascaris*. According to this author, the spermatozoon penetrates at any point of the surface of the egg, and Van Beneden's statements as to the existence of a micropylar orifice are represented as entirely incorrect. "It is very remarkable, however," says Zacharias (10, p. 143), "that in the great majority of cases only a single spermatozoon copulates with the egg of *Ascaris*. *If no predetermined point of impregnation is present, it is wholly inexplicable that only one of the many hundred spermatozoa which surround the egg in the upper part of the uterus should become attached to it.*" The idea that the receptivity of the egg is self-regulating does not seem to have occurred to Zacharias.

A very striking and convincing proof of attraction at a distance, and at the same time a confirmation of the interpretation above given to Selenka's observations, is found in the fecunda-

tion of the Lamprey egg, as described by A. Müller (24) and by Kupffer and Benecke (20). The micropylar area is represented by a watchglass-shaped segment of the egg membrane, and is surmounted by a prominent hyaline dome, which here replaces the mucous envelope ("Schleimhülle").

The first point which is of special interest in the description is the fact that *the spermatozoa which come in contact with the mucous envelope do not try to penetrate it, while those which reach the dome* (Müller's "Flocke") *immediately take a direction radial to the "watch-glass."* The whole dome becomes so thickly beset with spermatozoa that it presents the "picture of a beard." A. Müller likened the appearance to that of iron filings arranged in a feathery tuft around the end of a magnet, and the comparison is fully indorsed by Kupffer and Benecke. The whole account impresses one very strongly with the fact that attraction is felt *before* contact with the vitellus, and that its influence is strongest at, possibly confined to, the micropylar area.

With reference to this point, Kupffer and Benecke remark: "The micropyle is, therefore, not an open passage, as it would appear to be from the statements and figures of Calberla, but merely a more permeable place. But it remains a mystery how this point is always hit by a zoosperm, unless one is permitted to assume that *the interaction between egg and zoosperms is more energetic in that radius which passes through the micropyle than in any of the other radii*" (p. 15).

The attraction manifests itself not only in the behavior of the spermatozoa, but also in a *contraction* of the egg, which shows itself in the ring-like space left between the rim of the "watch-glass" and the vitellus. A single spermatozoon, after it has penetrated the dome and placed itself in a position radial to the micropylar surface, is sufficient to induce the immediate retraction of the vitellus; but the intensity of this action increases with the number of spermatozoa. Kupffer and Benecke justly infer, therefore, "dass die Zurückziehung des Dotters nicht auf einer Contactwirkung, sondern auf einer Fernwirkung der radiär geordneten Zoospermien beruht" (p. 11).

The second point of interest is the fact that *all undulations*

24. AUG. MÜLLER. Beobachtungen ü. d. Befruchtungerscheinungen im Ei d. Neunaugen. *Verhandl. d. Königsberger phys.-ökonomischen Gesellschaft*, 1864.

of the tail cease as soon as the head of the spermatozoon penetrates fully into the egg membrane. Its further progress, then, is due not to any automatic movements, but solely to centripetal attraction. As Kupffer and Benecke put it, "Es wird angezogen."

This attraction is felt even before the undulatory movement ceases, as shown by amoeboid changes in the head. "Eine Welle erhebt sich am hintern Ende des Kopfes und läuft an demselben bis an die Spitze hin ab, dert sich kuglig zusammenballend, weicht dann wieder zurück, um in erneuetem Anlaufe abermals vorzudringen. Dieses Spiel kann sich minutenlang wiederholen. Ist der Kopf bis in die Nähe der inneren Oberfläche der Eihaut gelangt, so sendet derselbe häufig einen feinen Faden pseudopodienartig vor, der den Rest der Strecke durchsetzt" (pp. 13-14).

A third fact of great significance is the *elongation of the head of the spermatozoon during its passage through the perivitelline space.* The nearer it comes to the vitellus the more elongated it becomes, the increase in length amounting in the end to about one-third. These remarkable changes show the attractive influence of the egg quite as clearly as Fol's "cone of attraction" demonstrates such action for the spermatozoon.

Taken all in all, Kupffer and Benecke's paper is by far the most important contribution to the evidences of attraction at a distance that has yet appeared, and whoever doubts such action will do well to read carefully their work.

The explanation which they offer for the phenomena is not one that I can accept, in so far as it refers the attractive influence exerted upon the spermatozoon, not to the vitelline protoplasm, but to a centripetally moving egg-nucleus. The idea that this egg-nucleus owes its origin to an impulse given to the vitellus by the spermatozoa of course requires no refutation. The method of accounting for the centripetal movement of the egg-nucleus accords fully with the views presented in the foregoing pages. The reason assigned for the failure of more than one spermatozoon to effect an entrance is entirely untenable, as before pointed out (p. 241).

The "Leitband des Samens," described by Calberla (17, p. 458), in the egg of *Petromyzon*, is regarded by that author (p. 485) as the functional equivalent of the "cone of attraction" in *Asterias*, and by Van Beneden (9, p. 373) it is likened

to the "bouchon d'imprégnation" in *Ascaris*. Kupffer and Benecke deny that the "Leitband" has the functional importance attributed to it by Calberla, and hence they prefer to call it an "Axenstrang."¹ Allowing that it serves to guide the spermatozoon into the vitellus, as asserted by Calberla, it is difficult to see how it furnishes any evidence of attraction at a distance. It is simply one of many protoplasmic threads, left by the *contraction of the vitellus*, not a cone of attraction *rising from the vitellus* to meet the spermatozoon. Neither the observations of Calberla nor those of Kupffer and Benecke permit us to identify the "Leitband" with the "bouchon" in *Ascaris*. This "bouchon"² arises before impregnation and quite independently of any spermatic influences, while the Leitband arises merely as a secondary result of the action of the spermatozoa. In view of the fact brought out by Kupffer and Benecke, that the spermatozoa may penetrate at any point of the micropylar area ("watch-glass"), and follow any one of the protoplasmic filaments formed in this region, or even pass between them in order to reach the vitellus, we would suggest that the "bouchon"—if such a structure is present in the Lamprey egg—is represented by the discoid thickening (A. Müller's "Deckel des Urbläschens") of the thin protoplasmic mantle. This disc occupies the active pole, and in extent corresponds closely with the micropylar area. (Cf. Kupffer and Benecke's Fig. 7.)

Nothing has yet been described in the teleostean egg that could with certainty be said to function as a "cone of attraction." The observations which I have made on pelagic fish eggs in conjunction with Mr. Agassiz are soon to be published elsewhere, and I will not here anticipate the results further than to say that a careful study of surface-preparations and sections has revealed not the slightest trace of such a cone. I may say, however, that conclusive evidence has been found that the area of impregnation is, as might have been predicted from the existence of a definitely localized micropyle, a limited one, with boundary lines encircling the germinal pole. It is per-

¹The Axenstrang is not constant in *Petromyzon fluvialis* (Kupffer and Benecke, p. 18).

²There is no such structure according to Zacharias.

fectly certain that these eggs have a polar area, comparable with the disc of impregnation in *Petromyzon*.

In many teleostean eggs a well-defined germinal disc is present before impregnation takes place, and in such cases the micropyle is usually described as occupying the centre of the disc. Such a position would indicate, as van Beneden (9, p. 376) has pointed out, that the spermatozoon penetrates at a predetermined point. It is probable, however, that the micropyle is a little eccentric, and that its polar distance is variable within certain narrow limits,—within an area which may be called the *pole of impregnation*.

Prof. Kupffer (25) has discovered in the egg of the trout (thirty m. or more after fertilization) small discs distributed over the surface of the blastodisc, which he compares with the "disque polaire" described by Van Beneden in the egg of *Ascaris*.

In surface views and under a low magnifying power, these "Polscheiben," a dozen or more in number, appear as light flecks. "Diese Scheiben bestehen aus einer senkrecht gestrichelten hellen Substanz, die als Fortsetzung der dünnen Dotterhaut erscheint, gegen die Mitte zu an Dicke zunimmt und hier von einem Ppropfe der Keimsubstanz durchbohrt wird, über dessen Oberfläche sich die Dotterhaut nicht fortzusetzen scheint. An der Basis des Propfes sieht man jederseits zwei kleine Hohlräume, die Durchschnitte eines die Basis ringsum umziehenden Kanals" (p. 6).

Kupffer holds that the germinal protoplasm ("Ppropfe") filling the central perforation of one of these "Polscheiben" fulfils the function of the "bouchon d'impregnation" of the *Ascaris* egg; but he has failed to verify this by observation.

The occurrence of several polar discs is taken as an evidence that more than one spermatozoon is required to fertilize the egg. But such evidence can have very little weight so long as the polar discs themselves remain entirely problematical. Kupffer defends his interpretation on the ground that the discs are similar in structure to the polar disc of *Ascaris*. But Zacharias has given very strong reasons for believing that the "disque polaire" and its central "bouchon d'impregnation" are artificial

25. C. KUPFFER. Die Befruchtung des Forellenes. *Bayerische Fischerei-Zeitung*, 1886.

productions, and hence the argument from analogy falls to the ground. When Kupffer or any other investigator succeeds in showing, under perfectly normal conditions, that more than one spermatozoon is concerned in the formation of the male pronucleus, we shall be ready to concede that a case of polyspermatic fecundation has been established. Decisive evidence can be obtained in no other way than by tracing the history of the male pronucleus, and it is precisely in this direction that Kupffer's observations are most incomplete.

Kupffer claims also "Copulationshügel" and polyspermatic fecundation for the amphibian egg and the lamprey egg. He has seen the hügel penetrated by spermatozoa, but has failed to determine their fate, and thus left it entirely uncertain whether more than one of them is concerned in the act of fecundation. Through the investigations of Bembeke, O. Hertwig, Roux, and Born, the history of the male pronucleus in the amphibian egg has been very completely ascertained. Thus far no positive evidence has been produced to show that the male pronucleus is the product of several spermatozoa. The indications are plainly in favor of monospermatic fecundation. If several spermatozoa took part in fecundation we should expect to find a corresponding number of pigment paths. Referring to this point, Roux (4, p. 173) remarks: "Van Bembeke fand wiederholt mehrere solcher Pigmentstrassen im Ei, während ich in etwa 100 geschnittenen Eiern dies blos einmal beobachtet und dies Ei stammte vom Ende der Laichperiode, wo Abnormitäten sehr häufig sind. Meine Untersuchungen bestätigen also die Angaben von O. Hertwig und Born, dass normaler Weise blos ein Samenkörper in das Ei eindringt."

With reference to the place of penetration, O. Hertwig (1, p. 82) makes the following statement: "Bei *Rana temporaria* erfolgt der Eintritt des befruchtenden Spermatozoon in den Dotter stets *am schwarzen Pol zur Seite des schleierförmig ausgebreiteten Excretkörpers* auf der vom Eikern abgewandten Eihälfte." Roux admits that the spermatozoon generally enters the egg near the upper pole, but denies that this is invariably the case (4, p. 174).

